

## The Kinetics of Fermentations in Sourdough Bread Stored at Different Temperature and Influence on Bread Quality

Venturi F\*, Andrich G, Sanmartin C and Zinnai A

Department of Agriculture, Food and Environment (DAFE), University of Pisa, Italy

### Abstract

The fermentations induced by the utilization of sourdough in bread-making, are able to enhance the qualitative properties of the final dough, improving its volume, texture and flavor, so to obtain a bread characterized by high qualitative properties and able to retard its staling process.

In particular the working conditions adopted can deeply affect the ratio occurring between the populations of lactic acid bacteria and alcoholic yeasts of the sourdough and then also the productions of the related metabolites, which deeply affect the organoleptic characteristics and then also the quality of the final bread.

The effect induced on the microbial and chemical composition of the sourdough by different values of the storage temperature utilized (13, 19 and 27°C) between two successive refreshes (~ 24 hours), was evaluated to put in evidence the different sensory characteristics assumed by the corresponding breads. The sensory profiles of the obtained breads evaluated by the descriptive analyses, were carried on by a panel of trained assessors using a sensorial sheet specifically developed for this purpose and characterized by unstructured graphical intensity scales; the reliability of judgments obtained was evaluated by statistical analysis.

So it was possible to put in evidence the high degree of correlation occurring among microbiological and chemical data of the sourdoughs and the sensorial characteristics of the corresponding bread.

Among the three storage temperatures of the sourdough, 19°C appears to be able to ensure the best organoleptic characteristics to this particular bread.

**Keywords:** Sourdough; Bread; Storage temperature; Lactic bacteria; Yeasts

### Introduction

Worldwide bread consumption accounts for one of the largest consumed foodstuffs, with over 20 billion pounds (9 billion kg) of bread being produced annually. This demand has been driven by consumers' seeking convenient fresh products that provide a source of nutritional value. Consequently, freshness is a key component in consumer acceptability and choice of bread [1,2]. The use of sourdough is a technology of expanding interest for improvement of flavor, structure and stability of baked goods [3], because it can actively retard starch digestibility leading to low glycemic responses, modulate levels and bioaccessibility of bioactive compounds, and improve mineral bioavailability [4]. Sourdough is a mixture of flour and water fermented with lactic acid bacteria (LAB) and yeasts, which determine its characteristics in terms of acid production, aroma and leavening [5]. A slack flour dough is inoculated with microbial starter, "mother culture", which is constantly renewed in a cyclical way, using specified recipes and ripening conditions [6]. As a consequence, sourdough is a unique food ecosystem: it selects for LAB and yeasts which are adapted to the environment, and hosts highly specific microbial communities [7,8]. When used in optimized proportions, sourdough can improve volume, texture, flavor, nutritional value of bread and increase the shelf life by retarding the staling process and by protecting bread from mould and bacterial spoilage [7]. The changes in cereal matrix, potentially improving nutritional quality, are numerous. They include acid production, suggested to retard starch digestibility, and to adjust pH to a range which support the action of certain endogenous enzymes, thus changing the bioavailability pattern of minerals and potentially protective compounds in the blood circulation [9]. The action of enzymes during fermentation also causes hydrolysis and solubilisation of grain macromolecules, such as proteins (i.e.: gluten) and cell wall

polysaccharides. It has also been suggested that degradation of gluten may render bread better suitable for celiac people [4]. Some strains of lactobacillus bacteria, involved in the process of souring of dough, produce an enzyme that breaks down a protein to be toxic to people with celiac disease [10].

Several types of traditional Italian bread have in common a long-time sourdough fermentation step and are known for their peculiar nutritional and qualitative traits in comparison with bakery products obtained with bread making protocols based on the use of selected yeasts and shorter fermentation step [11] so that many of them has been awarded the designation POD (i.e. bread of Altamura) or PGI (i.e. breads of Genzano, Matera and Coppia bread from Ferrara) recognized by the EU agricultural product quality policy.

As with other food processing, the challenge in fermenting cereal raw materials lies in the ability to combine good sensory quality with demonstrated nutritional and health benefits. Some of the mechanisms to improve and enhance the nutritional effects of fermented cereal systems are dependent on adjustment of the acidity for optimal action

\*Corresponding author: Venturi F, Department of Agriculture, Food and Environment (DAFE), University of Pisa, Italy, Tel: +39 (0)502216631; Fax: +39 (0)502216631; Email: [francesca.venturi@unipi.it](mailto:francesca.venturi@unipi.it)

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of the enzyme system present. Other mechanisms may be directly linked to other metabolites produced by yeast and lactic acid bacteria (l.a.b.), and then the control of different metabolic routes in the fermenting organisms becomes a key issue [9,4].

The aim of this research activity was to investigate on lactic bacteria and yeasts metabolic activity when different storage temperatures were adopted during the sourdough leavening, to determine how they affect the chemical composition of the sourdough and the sensory characteristics of the corresponding bread. So it would be possible to ensure a constant quality with elements of tipicity and recognisability that make a product different from each other similar.

## Materials and Methods

### Sourdough bread production

Every day the sourdough utilized was refreshed by following the methodology generally adopted in Tuscan bakeries. In particular it was utilized soft wheat flour type (Giambastiani mill, Lucca, Italy, “Consortium of Promotion and Protection of Tuscan Sourdough Bread”), water (60% of used flour) and a part (30% of flour) of sourdough coming from the previous baking [12]. According to the methodology described in the EU Regulation for PDO (Protected Designation of Origin) “Pane Toscano” [13,14] sourdough consist of a portion of dough from a previous preparation which, kept in a suitable environment, undergoes a gradual process of fermentation and acidification.

When suitably refreshed this sourdough, called “the starter” (biga), is able to initiate the rising process when combined with new dough [13].

In detail, at each cycle of refresh, the following amounts of the involved components are used to prepare the starter (biga): 1 kg of soft wheat flour type 0, at least 500 mL of sterilized water, at least 200 g of sourdough coming from the previous back-sloping process. After the mixing, an aliquot of the biga is stored in controlled conditions for at least 8 hours to be utilized in the refresh process of following day, while the remaining portion is maintained at room temperature for at least 2, 30 hours before to be cooked [14].

In the laboratory tests, the following amounts of the three involved components were used in order to refresh the sourdough utilized: sourdough coming from the previous back-sloping process (75 g), soft wheat flour type 0 (250 g) and sterilized water (150 g). These ingredients were mixed for 20 min in a kneading machine. A sample (50 g) of this dough was collected to be analyzed, while the remaining aliquot was further divided into two different portions, the first (150 g) was stored inside a temperature controlled cell for about 24 hours to be utilized in the refresh process of following day, while a part (200 g) of the remaining portion was maintained at 30°C for 4 hours before to be cooked (30 min at 230°C) inside an automatic oven to produce the wished bread [12]. Three different values of storage temperatures (13°C, 19°C and 27°C) were tested, while, for every temperature analyzed, the refresh procedure and the related sample collection was carried on for about two weeks.

### Microbial analysis of sourdough

The sourdough utilized in this research was initially obtained by the Consortium for the Promotion and Protection of PDO “Pane Toscano” sourdough bread [13,14].

In order to follow the time evolution of microbial populations in the sourdough during the storage as a function of the working conditions adopted, during each experimental run several samples of sourdough were aseptically collected into sterilized jars and viable and cultivable populations of yeasts and lactic acid bacteria (CFU/g d.m.) were evaluated following ICC standard methods [15]: n° 146 for Yeasts (WL agar) and n° 147 for Bacteria (MRS agar modified) [12].

As reported in a previous paper [16], the microbial composition of the sourdough was already investigated: the Yeasts population was represented mainly by *Saccharomyces cerevisiae* strain, while several strains of Lactic Acid Bacteria were identified by DGGE analysis (*Lb sanfranciscensis*, *Lb brevis*, *Lb curvatus*, *Lb plantarum* and *Weissella confusa*).

### Chemical characterisation: sourdough and cooked bread

Concentrations of the main fermentative metabolites (ethanol, D/L - lactic acid) produced in the sourdough during the storage time and in the bread samples after cooking, were determined by using specific Enzymic Kits (Megazyme), after pre-extraction with HCl 0,1N [12].

### Sensorial analysis of bread (crust and crumb)

Descriptive analyses was used to determine the sensory profiles of the bread samples. A panel of trained assessors evaluated bread samples 2 hours after to be taken out of the oven. The crumb separately from bread crust were tasted to better identify the specific contribution of these two bread fractions.

A 20 g portion of each sample, including 10g of crust and 10g of crumb, was presented to assessors in 3-digit coded glasses covered with a glass cover; 10 min intervals were allowed between each sample. All samples were assessed in duplicate. For evaluation, each assessor was provided with filtered water and un-salted crackers and asked to cleanse their palate between tastings. In addition, assessors received a list of attributes that included definitions to aid in their assessments [1,17]. Sample attributes were scored on unstructured 100 mm line scales labeled from low at 5 mm to high at 95 mm intervals. For each attribute (Table 1), ratings on the unstructured line scale were measured geometrically to produce intensity values [18].

Sensorial evaluation of bread crumb			
Aspect	Smell	Taste	Structure
Intensity of color	Grain	Sweet	Elasticity
Percentage of white	Acetic Acid	Salty	Compressibility
Density	Hay	Acid	Deformability
Porosity	Yeast	Bitter	Resistance to chewing
Structure regularity	Rancid	Grain Aroma	Surface Moistness
Homogeneity	Frank	Hay Aroma	Compactness
		Yeast Aroma	Cohesiveness
		Astringent	Juiciness
		Aftertaste	
Sensorial evaluation of bread crust			
Aspect	Smell	Taste	Structure
Intensity of color	Intensity of smell	Sweet	Structure regularity
Regularity of color	Cereals	Salty	Hardness
Tonality of color (yellow/brown)	Roasted	Acid	Friability
	Burned	Bitter	Crispness
	Fragrant	Cereal Aroma	Resistance to detachment crust/crumb
		Toasted Aroma	Amount of residual crumb after detachment
		Burned Aroma	
		Astringent	
		Aftertaste	

**Table 1:** Sensorial descriptors utilized to describe separately the crumb and the crust of the bread.

## Statistical analysis

To evaluate the statistical significance of the data obtained, the chemical and sensorial evaluations were performed in duplicate, the microbiological ones in triplicate. Statistical analysis of data was performed by one-way ANOVA (CoStat, Cohort 6.0), and means separation by the Tukey's HSD test at  $P \leq 0.05$  of significance.

## Results and Discussion

### The effect of the storage temperature adopted on microbial evolution in sourdough

To evaluate the effect induced by the main working variables (temperature, number of refreshes, gas composition of surrounding atmosphere), it was adopted a rigid experimental protocol changing only a parameter one at a time. So, according to the protocol previously reported (see Materials and Methods), the effect of the storage temperature of sourdough employed in the refresh process, was evaluated in laboratory scale so that all the other working variables could be more efficiently controlled. Figures 1a and 1b report the evolutions of the concentrations (CFU/g d.m.) of yeasts and lactic acid bacteria as a function of storage time of the sourdough at the analyzed temperatures (13, 19 and 27°C).

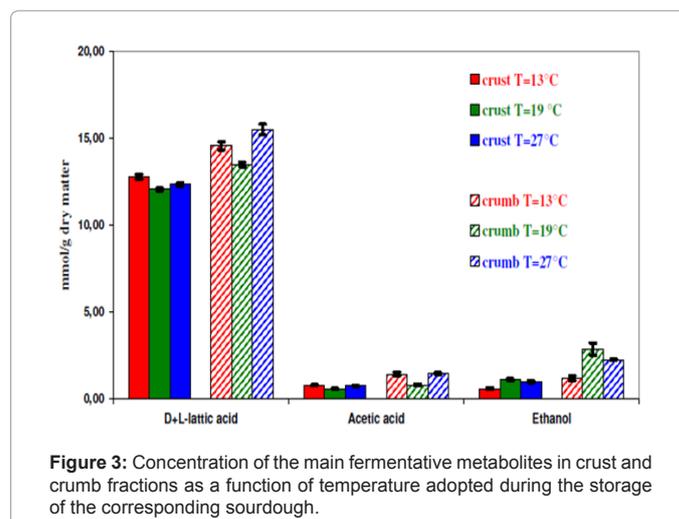
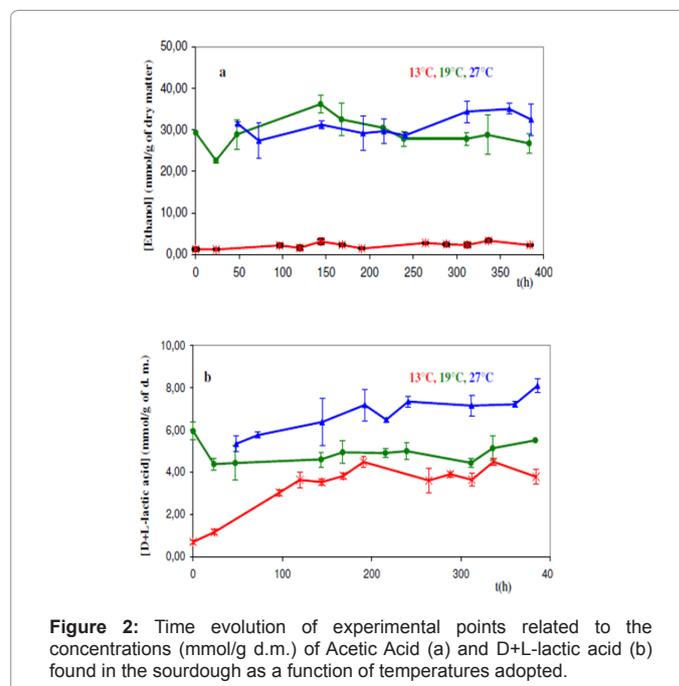
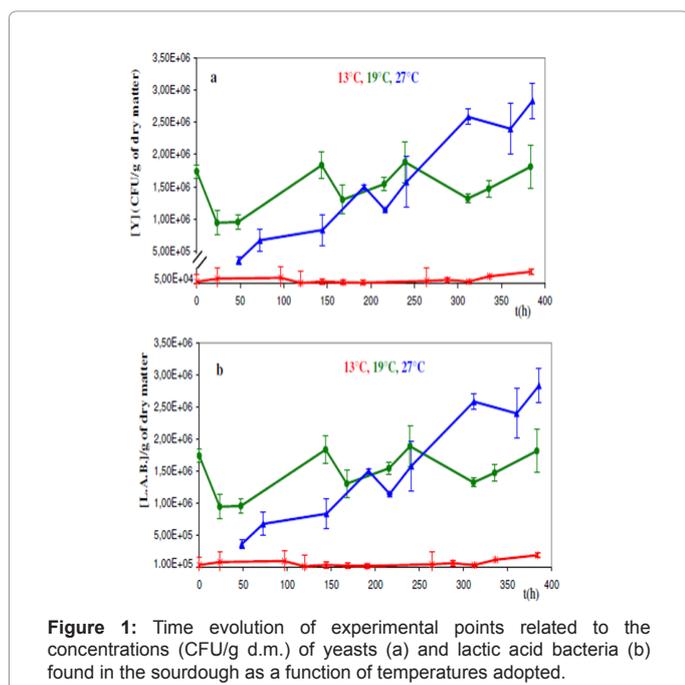
While for yeasts (Figure 1a), the use of the lowest temperature (13°C) determines an insufficient rate of growth, at the intermediate temperature (19°C) the concentration of viable cells increases and reaches the maximum value at the highest temperature (27°C). On the contrary, bacteria (Figure 1b) appear less sensible to the lowest temperature than yeasts, and over time they reach very similar values at all the temperatures adopted.

### The influence of storage temperature adopted on the production of the main microbial metabolites in the sourdough and in the cooked bread

**Sourdough:** By changing the storage temperature of sourdough, the yeasts concentrations varied such as those of their metabolites,

which are able to deeply affect the characteristics of the final bread. In particular, the concentration of ethanol in the sourdough (Figure 2a) was too low at the storage temperature of 13°C, so that the leavening process, due to carbon dioxide, which is produced at equimolar amount of ethanol by yeasts, could be considered not well developed. Although the concentration of lactic acid bacteria was similar over time at the different temperatures studied, the lactic acid production seems to be higher when temperature increases. So at 27°C, the specific activity of l.a.b. appears higher than at the other temperatures adopted (Figure 2b).

**Cooked bread:** As a consequence of the influence of storage temperature on the microbial and chemical composition of sourdough, it was possible to put in evidence that when the sourdough was stored at 19°C also the corresponding bread (crust and crumb fractions) was characterized by lower concentrations of acidic compounds (D+L-lactic acid and acetic acid) and higher concentration of ethanol (Figure 3)



than the breads obtained by the other sourdoughs. The differences are more evident in the crumb fraction than in the crust one.

### The influence of storage temperature adopted on sensorial expression of bread

By changing the storage temperature of the sourdough, yeasts and lactic acid bacteria metabolites varied greatly, affecting deeply the organoleptic characteristics of the cooked product. Because the attributes of the view and the smell are greatly influenced by the working conditions adopted during cooking process, which were the same for all the bread samples tasted, the differences in sensorial expression of the breads obtained by the three different sourdoughs were mainly related to the attributes of the taste.

In Figures 4a and 4b, the spider plots of the attributes of the taste, respectively related to crust and crumb of the breads obtained by the sourdoughs stored at different temperatures, are reported: when lactic acid bacteria and yeasts were present in a good concentration (bacteria/yeasts  $\approx 10/1$ ), the bread obtained was well leavened with a concentration of acidic compounds more tolerable by consumers. In fact, as shown in Figures 4a,4b, the sensorial analysis of the breads obtained in the different experimental runs of baking, produced the best results when sourdough was maintained at 19°C (sweet, low acidity and aftertaste, more elevated wheat and yeast flavours of crumb fraction as well as higher expression of aromatic components in crust fraction). On the contrary, when the temperature of sourdough storage was 13°C, yeasts were present at too low concentration, so that the bread obtained was not enough leavened and characterised by an unpleasant acid perception due to the reduced value assumed by the ratio lactic bacteria/yeasts ( $\approx 100/1$ ).

In synthesis, the evaluation of the overall pleasantness (Figure 5) associated to the three different bread produced, indicated that the best

sensorial profile was attributed to the sample of bread obtained by the sourdough stored at 19°C.

### Conclusions

Although some studies was already carried out to correlate microbial metabolites of many sourdoughs to the working conditions adopted [5-9], no information were still available concerning the typical Tuscan bread production.

Because the storage temperature adopted during the sourdough leavening deeply affects not only the chemical composition of sourdough but also the sensorial characteristics of the bread, some chemical indexes (Ethanol/D+L-lactic acid; Ethanol/ (D+L-lactic acid + Acetic acid); D+L-lactic acid/Acetic acid) were calculated by the ratio of the concentration of the main microbial metabolites determined in both crust and crumb fractions of Tuscan sourdough bread. Among the indexes which was calculated, the ratio D+L-Lactic acid/Acetic acid (Table 2), that could represent a good marker of activity of homo/hetero lactic bacteria fermenting strains, has been the best index able to provide some reliable indications about the quality of the bread. In particular, when the temperature utilized for the storage of sourdough was too low (13°C) or too high (27°C) this index showed values very close to each other and significantly lower than that showed by the crumb and the crust of the bread obtained by the sourdough stored at 19°C and characterized by the best sensorial profile.

The use of this new approach based on the integration of the information coming from both kinetic, chemical and sensorial data, has made possible the identification of the best operative conditions which could be adopted during Tuscan sourdough storage. The same experimental procedure make it possible to evaluate the effect induced by the other main working variables (number of refreshes, gas composition of surrounding atmosphere, etc.) on the properties of the

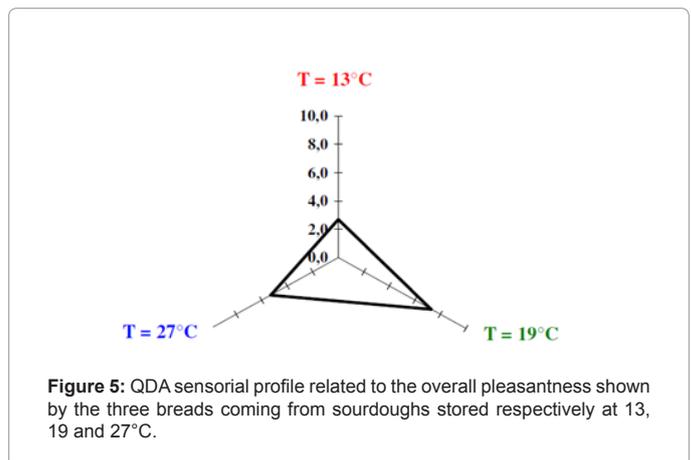
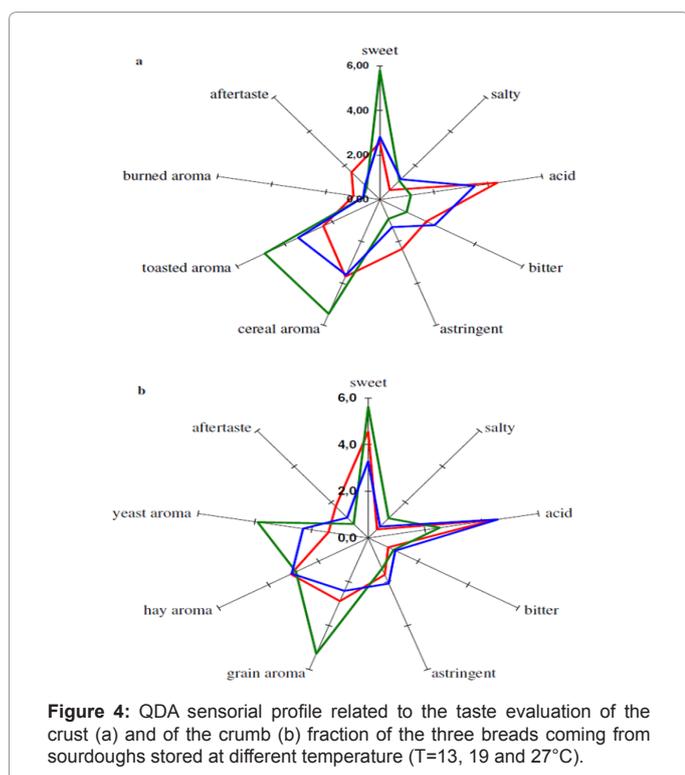


Figure 5: QDA sensorial profile related to the overall pleasantness shown by the three breads coming from sourdoughs stored respectively at 13, 19 and 27°C.

Sample	Ethanol	Ethanol	D+L-Lactic ac Acetic ac
	D+L-lactic acid	D+L-Lactic ac + Acetic ac	
Crust (T=13°C)	0.05	0.003	15.97
Crust (T=19°C)	0.09	0.007	20.25
Crust (T=27°C)	0.08	0.006	15.87
Crumb (T=13°C)	0.08	0.005	10.16
Crumb (T=19°C)	0.21	0.015	16.57
Crumb (T=27°C)	0.15	0.009	10.50

Table 2: Chemical indexes obtained by the chemical markers determined in crust and crumb fractions of the different breads.

final product. So it would be possible not only to obtain the sourdough bread characterised by the most favourable chemical and sensory properties but also to identify markers/parameters able to protect this product against fraud and imitation.

This experimental study represent part of a research project which was developed with the aim to obtain the PDO appellation for Tuscan sourdough bread [14].

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